Fine Structure of HIV and SIV

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Aim and Contents of this Note

This note, which is especially directed to scientists working in the fields of molecular genetics and immunology, summarizes essential aspects of morphogenesis and fine structure of HIV and SIV. All retroviruses, including HIV and SIV, show three functional states during their extracellular life cycle: assembly, maturation and aging. These states have been elucidated by transmission electron microscopy (TEM) and closely correlated to viral functions using wild-type virus as well as a variety of functional mutants (for review see Gelderblom, 1991; Hunter, 1994; Nermut and Hockley, 1996). In addition, two early steps in virus-cell interaction, virus adsorption and entry, have been studied by EM.

The possibility of clearly distinguishing between three morphologically and functionally distinct states is unique for retroviruses. Because of the close relationship between viral structure and function, EM studies have contributed key information to the understanding of HIV and, ultimately, to the development of antiretroviral strategies.

This note covers three topics. In Section I, techniques applied in the EM analysis of retroviruses are presented together with an overview of comparative retrovirus classification using both morphological and genetic information. In Section II morphogenesis and maturation of HIV/SIV are discussed in detail. Section III outlines the structure and composition of both mature and aging virus particles and thereby also covers the presence and possible role of host cell components with the virion.

I. Electron microscopy and morpho-classification of retroviruses

Electron microscopical techniques The majority of structural findings on HIV and SIV have been collected by TEM analysis of positive-contrasted thin sections of specimens, usually virus-producing cells (see Figs. 4–6). The specimens are fixed using aldehyde and osmium-tetroxide, dehydrated, and embedded in resins. Sections of 40 to 60 nm in thickness are post-stained using heavy metal salt solutions (for details see *Electron Microscopy* by Bozzola and Russel, 1991). The "positive" contrast observed in thin section EM is mainly due to the heavy metals bound to the specimen and their scattering power, i.e., the distribution of heavy elements is shown rather than the details of the object itself (Figs. 1, 5).

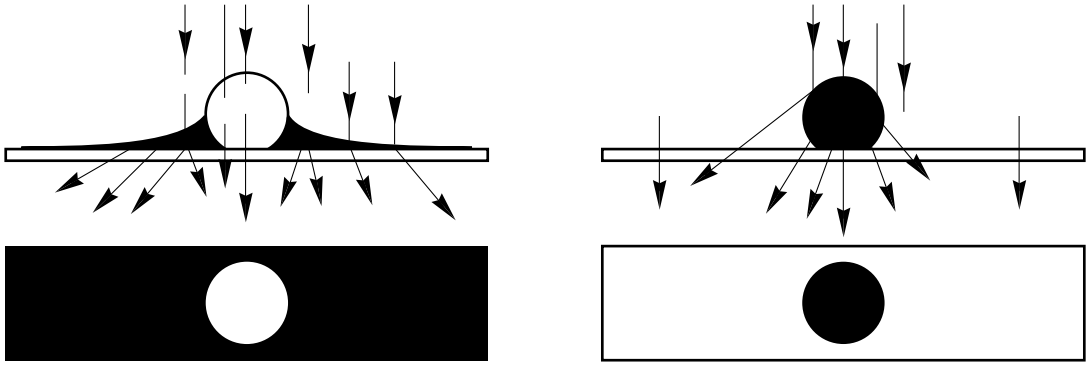

Fig. 1. Demonstration of positive and negative contrast phenomena in electron microscopy of biological specimens. Positive contrast is achieved by incubation with electron-dense heavy metal ions and covalent binding of this stain. In negative contrast the same or similar stains are used to wrap the biological structures without interacting chemically with the object.

Thin section EM involves chemical fixation and complete dehydration of the specimen which definitely abolish any "natural" state of biological structures. Nevertheless, thin section EM has been very rewarding in the structural analysis of retroviruses, due to the reproducible differential positive contrast and the appreciable fine structure attained in spite of all the artifacts.

Cryo-EM, in contrast, developed during the last two decades, has already given "molecular" information in many instances (for review see Dubochet et al., 1988; Müller, 1992). Cryo-EM involves physical fixation, i.e. rapid freezing instead of chemical fixation, and the analysis of frozen-hydrated specimens at temperatures below –140°C. The image obtained in cryo-EM depends on the scattering of the biological matter itself, as there is no heavy metal staining involved, and the first results obtained have been rewarding (Nermut et al., 1993). Specimen preparation, operation of the cryo-EM and image processing are, however, technically demanding. The biohazard of working with cryo-fixed, i.e. "living" virus poses additional obstacles. Therefore, in a recent cryo-EM study on the organization of HIV, non-infectious, immature particles generated by recombinant techniques and by "therapeutic" antiviral treatment (Fuller et al., 1997) were investigated.

In negative staining, biological structures usually remain unstained but are surrounded by an electron-dense stain and therefore appear lighter, i.e., in negative contrast, compared to the dark background. As with positive staining, salts of heavy metals—tungsten, uranium, and molybdenum—are used to create contrast. The stain, applied as a one to two percent solution, surrounds the virus particle pre-adsorbed to the grid and dries out (Figs. 1, 5). This rapid technique can result in high resolution information and is used to study selected aspects of retroviruses, (e.g. the presence and shape of SIV glycoprotein knobs) and also in conjunction with immuno-EM (IEM) (Gelderblom et al., 1988; Grief et al., 1989; Hockley et al., 1988; Chrystie and Almeida, 1988, 1989). But, because of osmotic damage and other artifacts, negative staining is not recommended as the preferred EM procedure for the elucidation of retrovirus fine structure.

Finally, scanning EM (SEM) has been used to study retroviruses and their interaction with the host cell. Conventional SEM is well suited to the study of virus-infected cell cultures (Gelderblom et al., 1988; Hockley et al., 1988; Nermut et al., 1993). State-of-the-art high resolution SEM can resolve the shape of virus envelope knobs (Renz et al., 1995), however, TEM is mostly preferred in virus fine structural studies, because SEM information is limited to the viral surface.

Table 1 Morphological criteria for the differentiation of retroviruses

	Mammalian		Avian	Type D	HTLV-BLV	Lentivirus	Spumavirus
Criteria	type B	type C	type C	group	group	group	group
Morphogenesis							
Cores preformed in the cytoplasm	+	–	–	+	–	–	+
Core assembly concomitant with budding	–	+	+	–	+	+	–
Distance of core shell to prospective envelope	wide	wide	wide	wide	narrow	narrow	wide
Morphology of the mature virion							
Shape of the core capsid	isometric	isometric	isometric	cone	isometric	cone-to-tube	isometric
Site of core	eccentric	concentric	concentric	diagonal	concentric	diagonal	concentric
Length of the envelope projections	8–9 nm	5 nm	5 nm	5 nm	5 nm	9–10 nm	12 nm
Anchorage stability of envelope knobs	high	relatively high	relatively high	relatively high	low	low	low

Fig. 2. Using the morphological criteria listed in Table 1, retroviruses form seven clearly distinguishable groups of agents. The members of each particular group share specific biological properties (for detail see Gelderblom et al., 1985a; Gelderblom, 1991).

Fig. 3. Phylogenetic tree of 19 “prototypic” members of the retrovirus family (modified from Griffith et al., 1997). The genetic classification fits remarkably well the morphological grouping shown in Fig. 2.

Criteria used for the morphological differentiation of retroviruses Starting with the pioneering work of Wilhelm Bernhard in the late 1950s, TEM has been used to differentiate and classify newly detected retroviruses (Bernhard, 1960). Comparative studies on members of the different retrovirus groups have revealed well-defined structural differences that can be assessed either during budding and in the immature state or with the mature and aging virion. The distinctive criteria listed in Table 1 have often been used to assign “new” retrovirus isolates according to morphology into the proper genus (Fig. 2; for review see Gelderblom, 1991).

II. Morphogenesis and maturation of HIV and SIV

The extracellular “life” of a retrovirus consists of several states that can be studied directly by EM (for review see Gelderblom, 1991; Nakai and Goto, 1996; Nermut and Hockley, 1996).

1. The virus is formed at the plasma membrane by an ordered self-assembly of virus structural Gag and Gag-Pol precurser polyproteins (Pr55 gag, Pr155 Gag-Pol) together with genomic RNA.
2. After closure of the spherical ribonucleoprotein shell (RNP) the immature virion is released from the cell.
3. This particle undergoes a functional and morphological maturation as a consequence of proteolytic cleavage of the precursor proteins by the viral protease (PR). The cleavage occurs during assembly or after release of the immature particle.
4. The mature virion ages, rapidly losing infectivity.

Retrovirus-coded proteins are named according to function and localization within the virion by 2-letter acronyms (Leis et al., 1988). This nomenclature is widely applicable in comparative studies on enveloped viruses and is also used in this article (for details see Fig. 7). During maturation, Pr55

Gag is cleaved by the viral protease (PR) into the four major structural proteins of the mature virion (Henderson et al., 1988, 1992): membrane associated or matrix protein (p17 MA), capsid, (p24 CA), nucleocapsid (p10 NC), and the C-terminal core-envelope link (CEL, p6 LI) (Höglund et al., 1992).

The budding process HIV and SIV, like most retroviruses, self-assemble at plasma membranes (Figs. 4, 5, 6). As a first indication, an electron-dense patch appears directly underneath the lipid bilayer, while on the bud, on the outside of the bilayer, individual virus envelope knobs can be seen. The dense patch grows continuously by side-to-side assembly of the Gag- and Gag-Pol-polyproteins ultimately forming a spherical, electron-dense RNP shell. This immature core is surrounded by a tightly fitting lipid bilayer. High resolution EM of immature virions clearly shows lateral interaction of Gag-rods in the RNP (Hockley et al., 1988; Nermut et al., 1994; Fuller et al., 1997). The immature virion is densely studded with glycoprotein knobs. Only after complete sealing of the RNP core shell, does the concentrically organized immature virion separate from the plasma membrane and pinch off into the extracellular space (Gelderblom, 1991; Göttlinger et al., 1991). The immature virion is slightly bigger than the mature particle (120–140 nm versus 110–130 nm; Nermut et al., 1993; Gentile et al., 1994) and appears to be more rounded than the latter which often shows an angular outline. The angular shapes have led to the conclusion that SIV and HIV represent isometric, probably icosahedral, structures (Marx et al., 1988; Özel et al., 1988; Goto et al., 1990; Nermut et al., 1993, 1994; Nermut and Hockley, 1996).

Virus formation and release depend on a number of structural requirements. Very early, intact Pr55 gag was shown using recombinant techniques to contain all the information necessary for viral assembly (Gheysen et al., 1989; Wills and Craven, 1991). The authentic viral RNP core shell of HIV is formed from two proteins, Pr55 Gag and Pr155 Gag-Pol, co-assembled at a ratio of approximately 20 to 1. Virus formation also involves oligomerization of the polyprotein and essentially represents a very constrained process (Jowett et al., 1992; Nermut et al., 1994; Zhang et al., 1996). A few factors shall be mentioned here.

Requirements for a functional MA Two factors, co-translational myristoylation and a highly basic region of the N-terminal MA domain, which binds to phospholipids, are essential for the stable association of MA with the plasma membrane and the formation of infectious HIV (Göttlinger et al., 1989; Bryant and Ratner, 1990; Zhou et al., 1994). Other regions of MA determine the targeting of Pr55 Gag to the plasma membrane, the regular site of viral assembly (Fäcke et al., 1993; Yuan et al., 1993). MA functions are required for both the self-assembly of the Gag- and the Gag-Pol-polyproteins and the insertion of the viral Env protein into the prospective envelope (Yu et al., 1992; Dorfman et al., 1994 b). Significantly, MA is part of the viral pre-integration complex and acts in targeting this complex to the nucleus in quiescent cells, a process that permits the infection of non-dividing cells (Bukrinsky et al., 1993).

Properties of a functional CA The side-to-side assembly of the polyprotein rods depends on multiple protein-protein interactions. This process can be inhibited by the intercalation of short peptides derived from the respective domains (Niedrig et al., 1993). The core capsid is made up of p24 CA (Gelderblom et al., 1987a; Chrystie and Almeida, 1989). Domains mainly at the C-terminus of p24 CA are essential for proper assembly of the RNP shell (Zhang et al., 1996), while the N-terminal region is required for the formation of the mature, cone-shaped core. The middle region of CA containing the "major homology motif" is well conserved among different retroviruses and essential for the formation of infectious virions (Mammano et al., 1994). Finally, Pr55 Gag functions are required for the co-incorporation of Pr155 Gag-Pol into the growing RNP shell (Smith et al., 1993).

The role of p2 Between CA and NC a spacer of 14 amino acids (p2) is situated; another spacer, p1 is located between NC and p6 CEL (Henderson et al., 1992). P2 is readily detectable in lysates of infected cells, but virtually absent from cell-released virions. The C-terminal portion of CA including part of p2 is crucial for HIV morphogenesis. The spacer is required for the ordered assembly of the spherical RNP shell. The removal of p2 from the C-terminus of p25 CA occurs late during the controlled Pr55 and Pr155 protein cleavage kinetics and is essential for the transition of the spherical RNP shell into the cone-shaped core, i.e., for viral maturation (Göttlinger et al., 1989 and personal communication; Pettit et al., 1994; Kräusslich et al., 1995).

Fig. 4. Thin section EM of HIV-1 strain IIIB. Between cellular processes and villi, HIV particles reveal different stages in development. Among many mature virions—characterized by the cone-shaped cores (arrows)—are shown budding viruses (arrow heads), cell released immature virions, and one particle representing an intermediate state in maturation (double arrow). Magnification: $\times$ 45,000

Fig. 5. EM of HIV comparing negative staining (a), and thin section positive staining, (b). (a) The phage-like particles result from the deleterious effects of the negative staining procedure on the labile envelope of isolated HIV. At the bottom a single free cone-shaped core is seen, still partly wrapped by remnants of the viral envelope. (b) Part of Fig. 4 shown at higher magnification. The two mature HIV reveal core-envelope-links (CEL), lateral bodies (arrow heads) and loss of envelope knobs. Two immature virions are still densely covered with surface knobs, during or just after release from the cell. Magnification: $\times$ 120,000

The role of NC For effective encapsidation of the viral genome the NC domain interacts during assembly with the viral RNA via two zinc-finger-like motifs (Clavel and Orenstein, 1990; Gorelick et al., 1990; Bess et al., 1992). In addition, a functional NC is required for the effective assembly (Jowett et al., 1992).

The function of p6 The C-terminal portion of the Pr55 polyprotein forms p6 essential for the last step in viral budding (Göttlinger et al., 1991). Deletions in p6 prohibit both the closure of the spherical RNP shell and particle release. From fine structural studies, p6 was inferred to also form the core-envelope-link (CEL) (Figs. 5b, 7; Nilsson et al., 1992; Höglund et al., 1992).

III. The assembly of the viral envelope

The two viral glycoproteins SU (surface protein, Gp120) and TM (trans-membrane protein, Gp41) are proteolytically processed from the viral Pr160 Env precursor in the Golgi region (Hallenberger et al., 1992). The incorporation of the glycoprotein knobs depends on the N-terminal part of MA (Yu et al., 1992; Dorfman et al., 1994 b; Freed and Martin, 1996). In HIV and a number of other retroviruses (Pepinski and Vogt, 1984) evidence exists for a close proximity of the MA and TM (Bugelski et al., 1995; for review see Hunter, 1994). The membrane spanning region of TM can be replaced by that of

Fig. 6. (a–c) Three different stages in the formation and maturation of HIV illustrating schematically the side-to-side assembly of the Pr55 and Pr155 polyprotein precursor, the immature virion, and the structural rearrangement of the processed proteins in the mature virion. (d) Mature SIV sm particles with cone-shaped cores, lateral bodies and a relatively dense fringe of surface projections. When the glycoprotein knobs are cut tangentially, a trimeric outline of the knobs becomes evident. Magnification: $\times$ 120,000. (e, f) Immuno EM localization of HLA class I (beta 2 microglobulin) and of class II (HLA DR), respectively, on budding and mature HIV-1 (from Gelderblom et al., 1987b). Magnification. $\times$ 80,000.

Fig. 7. Diagram of murine type C oncogenic retrovirus and HIV-1 combining morphological observations and IEM findings (Frank et al., 1978; Gelderblom, 1991) . In contrast to other retroviruses, lentiviruses contain an additional C-terminal p6 domain. The definition of the CEL at the small base of the core (Höglund et al., 1992) is based on both morphological evidence (see Fig. 5b) and molecular genetic findings (Göttlinger et al., 1991), which suggest an essential role for LI p6 in finishing the budding process. Modified from Gelderblom et al., 1987a, Nermut and Hockley, 1996.

an unrelated TM protein without changing viral functions, and the C-terminus of TM appears to be dispensable (Mammano et al., 1994; Wilk et al., 1996). The 72 glycoprotein knobs inserted into the viral envelope form a regular T = 7 laevo pattern (Özel et al., 1988).

The knobs are oligomers of loosely connected TM-SU complexes. The degree of oligomerization, however, is not clear. From cross-linking and molecular-genetic experiments both trimer- and dimer-formation have been reported (Schawaller et al., 1989; Weiss et al., 1990). From morphologic studies a trimeric shape of the knobs became evident (Gelderblom et al., 1987a; Gelderblom et al., 1988; Grief et al., 1989).

IV. Structure and composition of the mature virion

Most probably starting during the assembly of the precursor proteins, Pr55 and Pr155 are cleaved sequentially (Pettit et al., 1994) into the proteins observed in mature HIV/SIV (Henderson et al., 1988, 1992). This processing is required for the rearrangement of the inner proteins which takes place only after release of the immature virion from the cell.

Constituents and organization of the viral core While MA in the mature virion remains tightly bound to the inner leaflet of the lipid-bilayer, the other Gag-proteins condense to form the cone-shaped core typical of members of the lentivirus family (Figs. 4–7; Gelderblom et al., 1987a). In addition, cleavage of Pr155 Gag-Pol liberates multiple copies of the three essential viral enzymes: reverse transcriptase (RT), integrase (IN), and protease (PR). A single virion contains about 1200 molecules of CA and 80 of RT (Layne et al., 1992). In addition, two accessory virus-coded proteins, Vpr and Vpx are present in amounts equivalent to Gag. Vpr is present in all lentiviruses, while Vpx is restricted to HIV-2 and SIV. The incorporation of Vpr depends on C-terminal domains of p6 (Paxton et al., 1993). Vpr and Vpx have been localized by IEM between the core shell and the viral envelope inside the virion (Liska et al., 1994). High resolution localization of these proteins, however, is still required as well as an IEM analysis to clarify the composition of the electron-dense masses (“lateral bodies”) along the axis of the cone-shaped cores (Figs. 6b, 7). The fine organization of the viral genome inside the core appears complex (Höglund et al., 1992; Takasaki et al., 1997).

In addition to virus structural and accessory proteins, a number of host cell constituents e.g., actin, ubiquitin, and cyclophilin A are incorporated into the virion. Interestingly, the latter binds specifically

to CA of HIV-1 and was shown to be necessary for the infectivity of HIV-1 strains of the M-group viruses (Franke et al., 1994; Thali et al., 1994).

Constituents of the viral envelope The lipids of the viral envelope are derived from the plasma membrane of the virus-producing cell during budding (Aloia et al., 1993). Besides SU and TM, a number of cellular proteins have been demonstrated to be present on HIV and SIV by immuno EM and other immunological techniques (Gelderblom et al., 1987b; Henderson et al., 1987; Hoxie et al., 1987; Arthur et al., 1992; Meerloo et al., 1993). For example, HLA class I or HLA DR (class II) proteins are tightly inserted during budding into the viral envelope (Fig. 6) and exceed in number the viral SU and TM. Besides masking the virion as a "wolf in sheeps clothing" and having an impact on and interaction with the immune system, these host proteins might also exert other biological functions when present on the virion. Effective purification systems for viruses free of host components are lacking (Bess et al., 1997; Gluschankof et al., 1997).

Virus aging and comparative aspects Because of weak SU-TM interactions, the viral knobs are shed spontaneously from the viral surface (Gelderblom et al., 1985b). For HIV-1 strain IIIB a shedding half-life of 30 hours was determined. This rate easily explains the rapid decay of viral infectivity of IIIB (Layne et al., 1992). The SU knobs on HIV-2 and SIV, however, and in particular on fresh HIV isolates, have been reported to be more tightly associated with the viral envelope.

Except for possible differences in anchorage stability of the knobs, no consistent morphological differences are apparent between HIV-1, HIV-2, and SIV (Palmer and Goldsmith, 1988; Gelderblom, 1991; Nermut and Hockley, 1996). Finally, it should be mentioned that the fine structural data have been collected by EM studies on viruses grown in cell culture. The validity of the structural model still has to be proven by the EM evaluation of infectious plasma HIV.

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